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BLOOD LEVELS OF CALCIUM AND INORGANIC PHOSPHORUS IN HEREFORD CATTLE[1]

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INTRODUCTION

A possible relation between fertility and the levels of calcium and phosphorus in the blood of cattle has been suggested by various investigators. Proof of such a relation is, however, dependent upon a definite knowledge of the normal levels of these minerals. Such levels have been found for some breeds of cattle, but there is no general agreement in the results reported. The study here presented was undertaken to determine such standards for Hereford range cattle as a preliminary to the larger study of the relation of calcium and phosphorus blood levels to fertility.

LITERATURE REVIEW

The calcium and phosphorus blood levels of cattle recorded in the literature clearly indicated that type of feed, age, and breed influenced the blood level of those minerals. No reference was found, however, to the influence of sex on these levels. For the most part published data were based on small groups.

Haag and Jones (*6*)[2] reported the phosphorus level of blood plasma for mature cattle to be 5.2 mg. per 100 ml. This level does not agree with one reported by Johnson (*7*), which is 4.33 mg. per 100 ml. of whole blood for dairy heifers and cows, a mixed age group. Johnson found no difference for milking cows and dry cows, or for different breeds.

Robinson and Huffman (*11*) obtained 5.87 mg. of phosphorus per 100 ml. of blood plasma, for a herd of beef cattle not homogeneous as to age, the youngest being about a year old. Palmer, Cunningham, and Eckles (*10*) found the average inorganic blood phosphorus of a small group of calves to be 7.26 mg. per 100 ml. of plasma.

Beeson et al. (*2*), working with growing and fattening beef calves, found 6 mg. of blood phosphorus per 100 ml. of plasma adequate. They studied a homogeneous age group, but the results cannot be compared with those for a beef-range group because of the different type of feed.

Malan et al. (*9*), in the Union of South Africa, found that beef heifers approaching 2 years of age showed a normal phosphorus level of 5 mg. per 100 ml. of whole blood. This was a homogeneous age group, on pasture supplemented with phosphorus.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 362.

Black et al. (*3*), working with a mixed age group, reported the point at which phosphorus deficiency existed in range cattle, used in the experiment at the King ranch in southern Texas, to be 4 mg. per 100 ml. of blood.

Knox et al. (*8*) found the phosphorus in wet cows of Hereford range cattle to be below 4 mg. per 100 ml. of plasma, except in a small group of dry cows and steers, in which the level closely approached 4 mg. per 100 ml. of plasma.

Haag and Jones (*6*) reported the calcium blood level for normal mature dairy cattle to be 9.99 mg. per 100 ml. of plasma. Allcroft and Green (*1*) gave the analyses of 139 normal dairy cows and reported the range for the blood-calcium level to be 8.65 to 11.65 mg. per 100 ml. of serum. However, no levels were given for range beef cattle.

A corresponding lack of agreement is evident for other phases of the investigation of blood phosphorus and calcium. Shohl (*14 p. 185*) found that when inorganic blood phosphorus was high blood calcium was low, and vice versa. VanLandingham et al. (*13*), on the other hand, reported that age and level of phosphorus intake were without effect upon the calcium content of the whole blood of growing dairy heifers. Greaves et al. (*5*) also stated that phosphorus intake had little effect upon the calcium content of yearling beef steers, but that there was a close correlation between phosphorus intake and the inorganic phosphorus of blood. Black et al. (*3*) reported a similar correlation between phosphorus intake and the inorganic phosphorus intake and the inorganic phosphorus of blood.

MATERIALS AND METHODS

A large herd of Hereford cattle running on a 70,000-acre pasture was available for this study. The cows bled in the experiment were at the end of lactation and the beginning of gestation. The cattle subsisted for 7 to 12 months of the year on the available pasture plants with supplemental feed during the winter months.

This Wyoming pasture lies east of the mountains at an altitude of 6,000 to 7,000 feet above sea level. The forage is composed for the most part of mixed grasses, chiefly grama, needle, and buffalo. The comparitively uniform altitude, topography, and flora of this area are considered of importance, since it is recognized that the phosphorus content of forage varies with the composition of the soil upon which it is produced and with the growing season.

The calcium and phosphorus content of the range forage was not determined; unless the person doing the sampling was very familiar with the range and grazing habits of the cattle, it would be difficult on so large a range to sample accurately the forage consumed. There was no clinical evidence in the herd to show that the intake of these minerals was inadequate.

For the purpose of this study the cattle were divided into four groups, based on sex and age: (1) yearling bulls; (2) herd bulls—those older than group 1; (3) 2-year old heifers; and (4) aged cows—those older than group 3. In stage of development the yearling bulls are comparable to the 2-year-old heifers.

Blood samples for analyses for calcium and phosphorus content were taken in connection with the routine care of the herd. Whenever cattle were being run through the chutes for such operations as change

of pasture, branding, spraying, or dipping, it was planned to take samples of jugular blood from every tenth animal going through the chutes. Owing, however, to the exigencies of such operations it was sometimes the ninth or even the eleventh that was sampled. The distribution may be considered as adequate for securing representative samples of blood.

The general procedure was to collect jugular blood in glass vials and use the serum for the calcium and inorganic phosphorus determinations. The analyses were begun within 8 hours of collection of the blood, and even when a large number of samples were being handled the analyses were completed within 24 hours.

Inorganic phosphorus determinations were made by the method of Fiske and Subbarow (*4*); calcium determinations were made by the method of Roe and Kahn (*12*); both were adapted to the Klett photoelectric colorimeter.

Precautions were taken to check the reliability of determinations by including, in a series of determinations, a check sample of known phosphorus and calcium content; this was done each time new reagents were made up. The check samples were prepared in approximately 10-percent trichloroacetic acid, in such a manner that 5-ml. aliquots were equivalent to blood filtrates containing 3.5 to 8 mg. of inorganic phosphorus per 100 ml. and 9 to 11 mg. of calcium per 100 ml.

Calcium and phosphorus were measured in terms of milligrams per 100 ml. of blood serum, for brevity expressed as milligram percent (mg. percent.)

A total of 560 determinations was made. Statistical analysis was used on the entire series, but only tables of statistics pertinent to this discussion are presented.

EXPERIMENTAL DATA

The normal blood inorganic phosphorus and calcium levels of cattle reported in the literature show little agreement largely because of the influence of age, feed, breed, and the small experimental groups of cattle used. In the work that follows, it was attempted to take into consideration these factors, and in addition to note the influence of sex on the normal blood-phosphorus and calcium levels in Hereford cattle raised in the same geographical location and of similar breeding.

Statistical studies of the blood-phosphorus analyses are presented in table 1 for the four general classes of animals under study. The groups were large enough to warrant considerable reliance upon the trends and differences which are indicated.

TABLE 1.—*Blood-phosphorus level of cattle Hereford expressed as milligram percent.*[1]

Class	$\bar{x}$	σ	$\sigma\bar{x}$	n
Yearling bulls	7.30	1.46	0.103	203
Herd bulls	4.76	.81	.099	67
2-year-old heifers	5.07	.78	.063	155
Aged cows	4.89	1.17	.101	135

[1] $\bar{x}$ = Arithmetic mean average; σ = the standard error of an individual observation; $\sigma\bar{x}$ = the standard error of a corresponding mean; n = the number of animals comprising the sample (number of determinations).

The differences between means of blood-phosphorus levels (table 1) of the four homogeneous classes are indicated in table 2.

TABLE 2.—*Comparison of blood-phosphorus levels in different classes of animals.*

Classes	Difference between means	σd [1]
Yearling bulls vs. herd bulls	[2] 2.54	0.14
Yearling bulls vs. 2-year-old heifers	[2] 2.23	.14
Yearling bulls vs. aged cows	[2] 2.41	.12
Herd bulls vs. 2-year-old heifers	[3] .31	.14
Herd bulls vs. aged cows	.13	.11
2-year-old heifers vs. aged cows	.18	.12

[1] σd=the standard error of an indicated difference.
[2] =Significance at the 1-percent level.
[3] =Significance at the 5-percent level.

The data presented in tables 1 and 2 show that the amount of phosphorus in the blood of yearling bulls was high as compared to that of other classes; that the phosphorus content of the blood of the males was greater than that of the females; and that the quantity of phosphorus diminishes with age in Hereford cattle. This relationship with age is stronger in the male animal.

Results of the blood-calcium analyses are presented in table 3 for the same four classes, and the differences between the means of the blood-calcium levels are given in table 4. The sample sizes for the calcium determinations were smaller than those employed in the phosphorus determinations, because a calcium deficiency seldom occurs in roughage-consuming animals, and the numbers were large enough to assign mean levels.

TABLE 3.—*Blood-calcium level of cattle Hereford expressed as milligram percent* [1]

Class	$\bar{x}$	σ	$\sigma\bar{x}$	n
Yearling bulls	10.46	1.63	0.238	48
Herd bulls	13.03	1.33	.256	27
2-year-old heifers	9.13	1.39	.158	78
Aged cows	9.52	1.81	.249	54

[1] $\bar{x}$=Arithmetic mean average; σ=the standard error of an individual observation; σx=the standard error of a corresponding mean; n=the number of animals comprising the sample (number of determinations).

TABLE 4.—*Comparison of blood-calcium levels in different classes of animals*

Classes	Difference in means	σd [1]
Yearling bulls vs. herd bulls	[2] 2.57	0.349
Yearling bulls vs. 2-year-old heifers	[2] 1.33	.287
Yearling bulls vs. aged cows	[3] .94	.344
Herd bulls vs. 2-year-old heifers	[2] 3.90	.302
Herd bulls vs. aged cows	[2] 3.51	.357
2-year-old heifers vs. aged cows	.39	.295

[1] σd=The standard error of an indicated difference.
[2] =Significance at the 1-percent level.

The complementary association of calcium and phosphorus in the blood is shown when the mean blood-calcium values (table 3) are compared with the mean blood-phosphorus values (table 1). For

the herd bulls, the blood-phosphorus value is significantly less, while the calcium content of the blood is significantly greater, than for the yearling bulls. The same tendency for these mineral contents of the blood to vary with age is shown in the results for aged cows and 2-year-old heifers. The tendency for blood phosphorus to diminish and blood calcium to increase with age seems to be more marked in the male than in the female. It should be remembered, however, that the blood-calcium level may not reflect the true picture of what is taking place; that is, a low calcium intake and the consequent withdrawal of calcium from the bone and other tissues for the blood. Hence, the calcium level of blood, when used as an index of the existing calcium picture, must be interpreted with caution.

A brief study of variabilities for the values of blood-calcium and phosphorus within the four classes of animals is shown in table 5.

TABLE 5.—*Variabilities of the blood-calcium and phosphorous levels in the different classes of animals* [1]

Class	C. V. (Ca)	σC. V. (Ca)	C. V. (P)	σC. V. (P)
Yearling bulls	15.58	1.63	20.00	1.03
Herd bulls	10.21	1.40	16.93	1.50
2-year-old heifers	15.22	1.25	15.95	.93
Aged cows	19.01	1.87	22.43	1.43

[1] C. V. = Coefficient of variability ($100\frac{\sigma}{\bar{x}}$); σC. V. = the standard error of the coefficient of variability.

The data presented in table 6 show the results of correlation studies upon samples from each homogeneous group under study.

TABLE 6.—*Correlation of the blood-phosphorous and calcium levels in the different classes of animals* [1]

Class	r	σr	n
Yearling bulls	−0.39	0.129	45
Herd bulls	−0.03	.192	27
2-year-old heifers	[2] −.25	.117	66
Aged cows	.01	.140	54

[1] r = Coefficient of correlation; σr = the standard error of correlation; n = the number of animals comprising the sample (number of determinations). [2] Significance at the 1-percent level.

The negative values for the correlation coefficient indicate the inverse association of calcium and phosphorous in the blood. The correlations for the herd bulls and for the aged cows are not significant, possibly owing to the fact that these classes of animals are heterogeneous as to age. This indicates that the calcium-phosphorus ratio is a variable concept depending on age and sex, as indicated by table 7.

TABLE 7.—*The blood-calcium and phosphorus ratio in different classes of animals.* [1]

Class	$\bar{x}$	$s_{\bar{x}}$
Yearling bulls	1.43	0.054
2-year-old heifers	1.80	.060
Aged cows	1.94	.090
Herd bulls	2.73	.083

[1] $\bar{x}$ = Arithmetic mean average; $s_{\bar{x}}$ = the standard error of a corresponding mean.

All differences between mean calcium-phosphorus ratios (table 7) are significant. There is no tendency for this ratio to be constant in a heterogeneous group of animals. That there is a strong tendency for blood phosphorus to diminish with age in both sexes is the principal conclusion.

SUMMARY

The normal blood levels of phosphorus and calcium for range Hereford yearling and herd bulls and for range Hereford heifers and aged cows have been determined. The blood levels reported are based on analyses of samples from 560 animals.

The normal levels for blood serum inorganic phosphorus in milligram percent were 7.30 ± 0.103, 4.76 ± 0.099, 5.07 ± 0.063 and, 4.89 ± 0.101 for yearling bulls, herd bulls, 2-year-old heifers, and aged cows, respectively.

The phosphorus level of blood was found to be greater in bulls than in cows. The quantity of phosphorus diminishes with age in Hereford range cattle, but this relationship with age is stronger in the male animal.

The normal levels for blood calcium in milligram percent were 10.46 ± 0.238, 13.03 ± 0.256, 9.13 ± 0.158 and 9.52 ± 0.249 for yearling bulls, herd bulls, 2-year-old heifers, and aged cows, respectively.

A complementary association of phosphorus and calcium has been found as shown by the fact that $r=0.39\pm0.129$ for yearling bulls, -0.03 ± 0.192 for herd bulls, -0.25 ± 0.117 for 2-year-old heifers, and 0.01 ± 0.140 for aged cows.

The calcium-phosphorus ratio in homogeneous groups is a variable concept depending on age and sex.

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EFFECT OF MATURITY ON THE CHEMICAL COMPOSITION AND DIGESTIBILITY OF THE STEMS AND LEAVES OF SWEETCLOVER HAY[1]

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INTRODUCTION

As a hay and pasture crop sweetclover is becoming increasingly important. Varietal tests have pointed to the Madrid (*Melilotus officinalis* (L.) Lam.) and to the Spanish (*M. alba* Desr.) varieties as excellent yielders under farming conditions in eastern Washington. Since tonnage yield alone is not a reliable criterion of value, however, studies of the chemical life history of the Madrid and of the digestibility of the Spanish sweetclovers were carried out in order to test the suitability of these two varieties for pasture and hay.

REVIEW OF LITERATURE

A brief review of the literature bearing on the composition and digestibility of sweetclover was given in a previous paper.[2] Hay of the Alpha 1 variety was found to contain 8.8 percent digestible crude protein and 46.13 percent total digestible nutrients in its first-year crop when cut at a height of about 36 inches, and 9.38 percent digestible crude protein and 47.18 percent total digestible nutrients in the second-year crop when cut for hay at the bud stage. Similar figures for the Common White variety were 10.21 percent digestible protein and 47.44 percent total digestible nutrients the first year, and 8.93 percent digestible protein and 41.94 percent digestible nutrients the second year.

PLAN OF INVESTIGATION

During the years 1939 and 1940 a study of the chemical life history of Madrid sweetclover was undertaken. Digestion experiments were also made with sheep fed samples of hay from first- and second-year Spanish sweetclover cut at definite stages of maturity. A height of approximately 36 inches seemed best for the first year, and the early

[1] Received for publication September 25, 1944. Published as Scientific Paper No. 621, College of Agriculture and Agricultural Experiment Station, State College of Washington.

[2] SOTOLA. DIGESTIBILITY OF NUTRIENTS IN FOUR VARIETIES OF SWEETCLOVER HAY. Jour. Agr. Res. 61: 887–891. 1940.

bud stage for the second year. Since field curing of sweetclover is usually accompanied by a large leaf loss, this study was designed to show what proportions of the digestible nutrients are found in the steams and leaves of the first and second year's growth. The plan provided for six separate digestion experiments in which stems, whole hay, and leaves of the first- and second-year crops were used separately during 10-day digestion experiments, each experiment being preceded by a 7-day preliminary feeding period.

METHODS OF SAMPLING AND ANALYSIS

CHEMICAL LIFE HISTORY OF MADRID SWEETCLOVER

Clippings of the new seeding were first taken when the plants were 7 inches high and thereafter at approximately 2-week intervals throughout the growing season. Different plants were selected each time and cut 1 inch above the ground line. Each clipping was made at approximately 9:00 a. m., and the samples were immediately taken to the laboratory, where they were air-dried, chopped, mixed, and smaller samples set aside for chemical studies. (Special precautions were taken to retain complete samples, avoiding leaf and other losses.)

The forage samples were subjected to the usual chemical analysis to obtain information as to the changes in the nutrient content that take place during the development of the plants.

The second spring all dead stalks of the previous year's growth were removed so as not to contaminate the new growth during the sampling operation.

DIGESTION EXPERIMENTS

Samples of 3,000 pounds each of the first- and second-year crop were carefully air-dried on canvas, and two-thirds of each sample was separated into stems and leaves. In separating the stems and leaves, all small petioles and blossoms were included with the leaves and all weeds were discarded. The samples were chopped into half-inch lengths and aliquots taken for chemical study. The daily feed requirement for each sheep was immediately weighed into paper bags to avoid discrepancies in dry-matter intake between trials arising from variations in humidity.

The detailed method of conducting digestion experiments with sheep by the use of metabolism cages has been previously described.[3] Official methods[4] of analysis were used for all chemical determinations.

EXPERIMENTAL RESULTS

CHEMICAL LIFE HISTORY OF MADRID SWEETCLOVER

Seedlings of Madrid sweetclover, 7 inches high, were sampled June 13, 1939. Sampling continued at intervals of about 2 weeks into October, at which time the plants were approximately 34 to 36 inches high.

[3] SOTOLA, J. RELATION OF MATURITY TO THE NUTRITIVE VALUE OF FIRST, SECOND, AND THIRD CUTTINGS OF IRRIGATED ALFALFA. Jour. Agr. Res. 35: 361–383, illus., 1927.

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The first samples of the second year's growth were collected on April 30 when the plants were 5 inches high, and samplings continued every 2 weeks until July 9, at which time the plants were mature and approximately 73 inches in height. The relation of stems to leaves as affected by the height and maturity of the plant is shown in table 1.

TABLE 1.—*Relation of stems to leaves in air-dried Madrid sweetclover plants*

First-year crop				Second-year crop				
Date	Height above ground	Leaves air-dried	Stems air-dried	Date	Stage of maturity	Height above ground	Leaves air-dried	Stems air-dried
	Inches	*Percent*	*Percent*			*Inches*	*Percent*	*Percent*
June 13	7.0	28.47	71.53	Apr. 30		5.0	71.88	28.12
June 27	11.0	37.02	62.98	May 14		16.0	63.44	36.56
July 11	15.0	55.02	44.98	May 28		38.0	37.10	62.90
July 25	22.0	53.48	46.52	June 11	Early bud	66.0	28.48	71.52
Aug. 8	30.0	63.31	36.69	June 24	Bud	73.0	27.80	72.20
Aug. 22	36.5	63.45	36.55	July 9	Flower	73.0	26.56	73.44
Sept. 19	35.0	63.89	36.11					
Oct. 3	34.0	66.33	33.67					
Oct. 17	34.0	67.46	32.54					

The proportion of leaves steadily increased with the height and maturity of the plant during the first year. However, the same plants sampled during their second year's growth contained a high proportion of leaves early in the season and became stemmy as the season progressed.

The first-year crop on August 22, when the plants were 36.5 inches high, contained 63.45 percent of leaves, while the second-year crop on May 28, when the plants were 38 inches high, contained 37.1 percent of leaves. The mature plant of the first year's growth had a leaf-stem ratio of 1:0.48 as compared with a leaf-stem ratio of 1:2.76 for the mature plant of the second year's growth. The second-year crop matured earlier in the season than the first-year crop, and it was stemmier.

COMPOSITION OF THE DRY MATTER OF MADRID SWEETCLOVER

In 1939 at 7 inches high the fresh sweetclover contained 82.20 percent moisture; at 11 inches, 78.16 percent; at 15 inches, 68.00 percent; at 22 inches, 67.07 percent; at 30 inches, 62.33 percent; and at 35.5 inches, 53.00 percent. After this growth stage the moisture percentage remained fairly stationary for the remainder of the season. Almost identical moisture percentages were observed during the second year.

The chemical changes in maturing stems and whole plants of Madrid sweetclover (table 2) showed a progressive decrease in protein, ash, and fat, and an increase in crude fiber. The leaves showed some decrease in protein but the rate of decrease was lower.

In the second-year crop the protein content of the whole plants decreased sharply while a marked rise in crude fiber was observed. The leaves again changed at a much slower rate in regard to both nutrients, the changes being in the same direction.

TABLE 2.—*Composition of the dry matter in Madrid sweetclover plants of the first- and second-year crop*

STEMS

First-year crop						Second-year crop					
Date	crude protein	Ash	Ether extract	Crude fiber	N-free extract	Date	Crude protein	Ash	Ether extract	Crude fiber	N-free extract
	Percent	*Percent*	*Percent*	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
June 13	10.76	11.43	4.45	21.02	52.34	Apr. 30	10.09	5.05	2.55	19.41	62.90
27	14.05	11.32	2.21	26.17	46.25	May 14	10.79	9.28	2.89	25.35	51.69
July 11	8.79	8.47	1.58	32.65	48.51	28	6.95	7.26	2.30	36.85	46.64
25	9.10	6.46	1.72	36.01	46.71	June 11	5.34	4.93	1.10	46.38	42.25
Aug. 8	8.44	5.50	1.35	44.70	40.01	24	4.34	3.85	.91	52.17	38.73
22	9.06	5.64	1.32	42.31	41.67	July 9	3.11	3.21	.82	53.70	39.16
Sept. 19	7.83	4.11	1.50	41.30	45.26						
Oct. 3	8.10	5.29	1.96	41.71	42.94						
17	7.73	4.10	1.44	41.78	44.95						

WHOLE PLANT

June 13	23.07	12.84	4.18	11.54	48.37	Apr. 30	27.24	12.51	5.14	10.93	44.18
27	19.42	10.97	1.55	17.55	50.51	May 14	22.04	11.35	3.72	14.00	48.89
July 11	15.59	9.46	2.96	22.20	49.79	28	14.94	8.77	3.29	25.45	47.55
25	17.24	8.24	2.97	23.67	47.86	June 11	9.62	6.11	2.41	36.57	45.29
Aug. 8	14.40	7.07	2.67	31.84	44.02	24	8.86	5.03	2.10	39.47	44.54
22	13.36	6.98	2.65	31.62	45.39	July 9	8.35	4.89	1.88	42.99	41.89
Sept. 19	12.05	5.90	2.55	31.88	47.62						
Oct. 3	11.61	5.83	2.43	33.31	46.82						
17	10.68	6.05	2.59	31.01	49.67						

LEAVES

June 13	28.33	11.41	3.49	10.56	46.21	Apr. 30	30.71	12.83	4.92	7.79	43.75
27	25.07	11.47	3.27	8.76	51.43	May 14	23.70	11.90	5.38	7.60	51.42
July 11	22.95	11.57	4.84	10.17	50.47	28	22.67	11.63	5.74	8.54	51.42
25	26.36	10.35	4.45	9.75	49.09	June 11	23.22	10.24	4.80	10.49	51.25
Aug. 8	23.47	11.70	3.46	9.55	51.82	24	19.71	9.87	4.07	13.48	52.87
22	20.99	10.43	4.54	9.97	54.07	July 9	21.48	8.60	2.56	14.81	52.55
Sept. 19	19.77	9.07	3.76	8.63	58.77						
Oct. 3	18.19	9.38	5.05	9.65	57.73						
17	17.93	12.00	4.40	9.71	55.96						

CHEMICAL COMPOSITION OF SPANISH SWEETCLOVER

The chemical composition of the forage samples fed during the digestion experiments is shown in table 3. The level of protein was higher in the stems and leaves of the second- than of the first-year crop, as was true also of the crude fiber. The stems of the second-year crop harvested at the early-bud stage contained 42.31 percent of crude fiber.

The leaves of the first-year crop contained 3.286 percent calcium in contrast to 0.599 percent found in the stems. The second-year crop was lower in calcium but slightly higher in phosphorus than the first-year crop. The value for calcium in sweetclover hay compares very favorably with that for good grade alfalfa hay reported in an earlier paper.[5]

[5] See footnote 2, p. 365.

TABLE 3.—*Chemical composition of Spanish sweetclover stems, whole plant, and leaves used in digestion trials with sheep*

FIRST-YEAR CROP (cut at 36 inches)

Sample	Moisture	Crude protein	Ash	Ether extract	Crude fiber	N-free extract	Calcium	Phosphorus
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Stems	12.90	6.05	4.07	1.07	36.41	39.50	0.599	0.230
Whole plant	10.76	9.95	6.64	1.18	26.98	44.49	1.734	.200
Leaves	17.39	15.40	9.22	2.67	9.11	46.21	3.286	.214

SECOND-YEAR CROP (cut at early bud stage)

Stems	7.04	10.25	6.73	1.30	42.31	32.37	0.582	0.372
Whole plant	9.11	16.58	10.20	2.04	30.41	31.66	1.285	.330
Leaves	8.19	24.88	12.62	3.28	14.88	36.15	2.073	.360

COEFFICIENTS OF APPARENT DIGESTIBILITY OF SPANISH SWEETCLOVER

The individual coefficients of apparent digestibility of Spanish sweetclover are shown in table 4. The sheep were fed at the same level of dry-matter intake, based upon the live weight of the sheep. Extreme care was taken to obtain an accurate partition of fecal and urinary nitrogen, and the nitrogen determinations were made on fresh feces in order to avoid any error that might arise from loss of ammonia in air-drying the feces. The feces, even in cool weather, were treated with dilute formaldehyde to prevent loss of ammonia from bacterial action.

TABLE 4.—*Coefficients of apparent digestibility of nutrients in the stems, leaves, and whole plant of Spanish sweetclover during its first and second year when harvested for hay and fed to wether lambs and yearlings*

STEMS

Lamb No.	First-year crop					Second-year crop				
	Dry matter	Crude protein	Ether extract	Crude fiber	N-free extract	Dry matter	Crude protein	Ether extract	Crude fiber	N-free extract
180	56.0	55.7	28.4	41.8	71.3					
188	51.7	49.7	38.9	38.4	67.4					
190	50.5	47.2	24.7	35.6	68.6	50.6	55.5	58.9	43.2	63.0
196	48.4	47.0	29.9	32.0	67.3	54.3	57.9	51.5	46.0	66.9
283						49.9	55.2	54.5	42.1	61.8
Average	51.6	49.9	30.5	37.0	68.6	51.6	56.2	55.0	43.8	63.9

WHOLE PLANT

180	59.1	64.3		49.3	75.0	53.1	70.4	48.9	36.4	60.5
188	58.8	65.7		46.7	76.7					
190	57.3	62.9		45.7	75.4	53.7	73.7	53.0	35.2	60.1
196	58.2	62.8		46.9	74.0	53.2	73.4	57.6	35.2	61.3
283						53.2	70.5	55.5	34.1	61.5
Average	58.4	63.9		47.2	75.3	53.2	72.0	53.8	34.7	60.9

LEAVES

180	63.7	75.2	5.0	41.4	77.3	61.6	75.5	38.7	41.6	69.7
188	66.1	77.5	8.0	45.1	79.2					
190	62.9	75.3	9.8	31.9	77.5	59.3	77.4	42.8	32.2	66.9
196	64.8	76.2	5.1	47.7	78.3	64.4	79.0	43.2	43.7	72.0
283						64.4	78.5	43.6	44.2	73.8
Average	64.4	76.1	7.0	41.5	78.1	62.4	77.6	42.1	40.4	70.6

The wether lambs in these digestion experiments ranged from 37 to 59 kg. live weight and consumed a daily average of 15 to 19 gm. of dry hay per kilogram of live weight.

It may be observed in table 4 that there was practically no difference in the average digestibility of the dry matter in the stems of the first- and second-year crops. Likewise the dry matter of the whole hay of the first- and second-year crop and the dry matter of the leaves of the first- and second-year hay were quite similar. However, the dry matter of the leaves was almost 25 percent more digestible than that of the stems.

The average digestibility coefficient for crude fiber of the whole plant was 47.2 percent in the first-year crop and only 34.7 percent in the second-year crop, a difference of 36 percent in favor of the first-year crop.

The values of 76.1 and 77.6 as coefficients of digestibility of the protein in the leaves of the first- and second-year crop were very high, showing leaf protein on the average for both crops to be 45 percent more digestible than the protein of stems. Furthermore, leaves were much higher in protein than stems (table 3). The desirability of curing hay with a high leaf percentage is obvious from these figures.

DIGESTIBLE NUTRIENTS OF SPANISH SWEETCLOVER

The digestible nutrients in the stems, whole plant, and leaves of Spanish sweetclover are shown in table 5.

Assuming first-year sweetclover hay of the Spanish variety containing 52.59 percent of total digestible nutrients to have a value of 100 percent, the second-year crop would have a value of 84.1 percent on the basis of digestion experiments with sheep. On the basis of percentage composition, the stems of the first-year crop contained as much total digestible nutrients as the whole hay of the second-year crop, but only 25.8 percent as much digestible crude protein. Likewise, the leaves of the hay from the first-year crop contained only 60.7 percent as much digestible crude protein as the leaves of the second-year crop.

TABLE 5.—*Digestible nutrients in the stems, whole plant, and leaves of Spanish sweetclover as determined with wether lambs for the first- and second year-crops, based on average coefficients of apparent digestibility*

Sample	Dry matter	Crude protein	Ether extract	Crude fiber	N-free extract	Total digestible nutrients	Nutritive ratio [1]	Total digestible nutrient rating [1]
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*		
FIRST-YEAR CROP								
Stems	44.94	3.02	0.33	13.47	27.10	44.33	13.68	84.3
Whole plant	52.12	6.36	----------	12.73	33.50	52.59	7.27	100.0
Leaves	53.20	11.72	.19	3.78	36.09	52.02	3.44	98.9
SECOND-YEAR CROP								
Stems	47.97	5.76	0.72	18.53	20.68	46.59	7.09	88.6
Whole plant	48.44	11.94	1.10	10.55	19.28	44.25	2.71	84.1
Leaves	57.29	19.31	1.38	6.01	25.52	53.94	1.79	102.6

[1] 1 to first-year whole hay 100 percent.

The second-year hay contained 64.8 percent more digestible crude protein than the first-year hay. According to previous work,[6] sweetclover hay from plants of Common White sweetclover harvested at similar stages of maturity contained 10.21 and 47.44 percent of digestible protein and total digestible nutrients respectively in the first-year crop, with 8.93 and 41.94 percent in the second-year crop. Thus it is apparent from table 5 that the Spanish variety is superior both in the availability of its nutrients to sheep and its production of dry matter.

SUMMARY

Plants of Madrid sweetclover steadily increased in leaf percentage as they matured during the first year, but decreased at an even greater rate in leaf percentage during the second year. The plants decreased in protein, fat and ash and increased in crude fiber as they matured during the first year. As the second-year growth progressed the plants decreased sharply in protein and increased in crude fiber, much more so than during the first year. The leaves in both first- and second-year crops showed some decrease in protein, but the decrease was much less than that in the whole plant.

Digestion experiments in which sheep were fed Spanish sweetclover showed the first-year crop, cut for hay when 36 inches high, to contain 6.36 percent digestible crude protein and 52.59 percent total digestible nutrients. The second-year crop, more fibrous and stemy, contained 11.94 percent digestible crude protein and 44.25 percent total digestible nutrients.

Leaf protein, on the average, was 45 percent more digestible than protein of the stems in both the first- and second-year crops of Spanish sweetclover.

[6] See footnote 2, p. 365.

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